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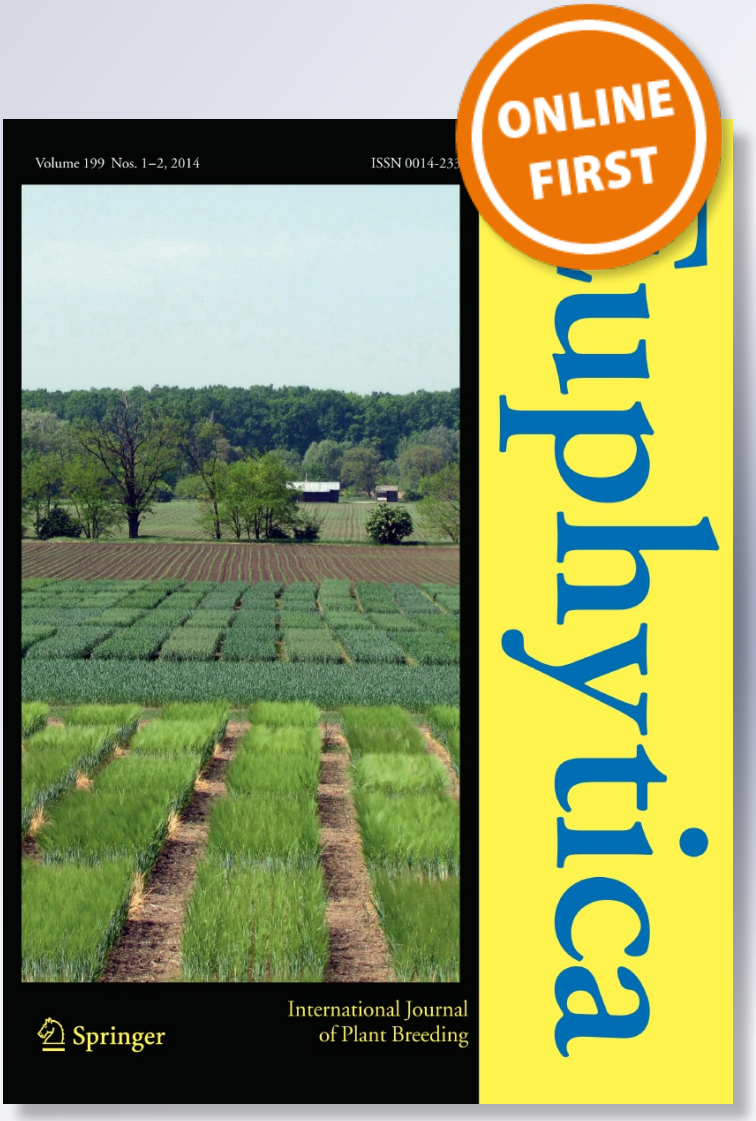

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Abstract The salt stress responses of wheat–barley addition lines (2H, 3H, 3HS, 4H, 6H, 7H and 7HL) were compared to those of the parental genotypes wheat cv. Asakaze and barley cv. Manas and two other wheat genotypes [Chinese Spring (CS) and Mv9kr1] during germination and in young plants grown in hydroponic culture with or without salt treatment. Among the wheat genotypes frequently used for interspecific hybridization, Asakaze possesses relatively high salt tolerance, as indicated by the less pronounced reduction in germination % and in root and shoot growth and the retention of high leaf water content and photosynthetic activity, as compared to CS and Mv9kr1. The barley cv. Manas showed better salt tolerance than wheat cv. Asakaze, although Manas accumulated more Na in the root, but its transport to the shoots is restricted. Among the addition lines tested, the disomic addition line 7H and ditelosomic line 7HL exhibited higher salt tolerance both during germination and in the early developmental stages than the wheat parent, which may be related to the elevated osmotic adjustment capacity of these addition lines, similar to that found for barley cv. Manas. The paper also discusses the effects of other chromosomes on the salt stress response.

Keywords Osmotic stress · Photosynthesis · Salt tolerance · Wheat–barley addition lines

Introduction

Both wheat and barley are extensively cultivated cereals but differ in their pathogen responses, nutritional quality and adaptation to environmental stresses. Interspecific hybridization between these species makes it possible to transfer agronomically useful traits, such as earliness, sprouting resistance and various traits responsible for nutritional quality (e.g., β-glucan content) (Islam et al. 1978; Islam and Shepherd 1990; Koba et al. 1997; Molnár-Láng et al. 2014). However, there are fewer examples of the successful improvement of tolerance to abiotic stress factors, such as drought or salt, probably due to the multigenic nature of these traits (Colmer et al. 2006; Hajjar and Hodgkin 2007; Molnár et al. 2007; Dulai et al. 2010).

Salt stress is one of the most widespread abiotic constraints in food production, occurring on saline

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soils and on irrigated lands mainly due to unsuitable water management or high evaporation. Depending on the salt concentration, the length of exposure, the stage of plant development and the environmental conditions, salt stress may induce physiological malfunctions, such as osmotic, ionic and various secondary (e.g., oxidative) disorders (Zhu 2001). Osmotic stress, as a primary reaction triggered by relatively moderate salinity levels, decreases soil water potential, reduces water uptake and may cause cell dehydration, leading to limited growth, stomatal closure and a decline in the photosynthetic activity of the leaves. Salinity may also cause ionic stress when ions are taken up by plants at an above-optimum concentration, disturbing the integrity and selectivity of the root plasma membrane and the homoeostasis of essential ions. Intense salt stress may trigger the acceleration of senescence, enzymatic and metabolic dysfunctions, including a decline in photosynthetic CO_2 assimilation and electron transport activity, chlorophyll degradation, the accumulation of reactive oxygen species and membrane damage, leading to plant death during prolonged exposure (Mahajan and Tuteja 2005).

Since salinity above the optimum level may also substantially reduce the biomass production and yield of crop plants, the development of salt-tolerant plants is of increasing economic importance. Although both wheat and barley show wide diversity in their response to salt stress and represent different mechanisms for salt tolerance, barley is usually regarded as being a more salt-tolerant cereal than bread wheat (Munns et al. 2006, 2011). In barley, major QTLs controlling salt tolerance co-localized with QTLs for other traits, such as biomass growth, chlorophyll content and leaf senescence, and controlling the contents of various ions including Na^+, were recently shown on chromosomes 6H and 4H (Long et al. 2013). The QTL on 4H might relate to the HKT1;5 gene encoding a Na^+-selective transporter located on the plasma membrane of root cells surrounding xylem vessels, which is therefore ideally localized to reduce Na^+ transport to the leaves (Munns et al. 2012). Another association mapping QTL for Na^+, K^+ and the Na^+/K^+ ratio, located on chromosome 7H, might be related to the HvNax3 locus controlling shoot Na^+ exclusion (Shavrukov et al. 2010). Using disomic addition lines involving wheat cv. Chinese Spring and barley cv. Betzes, Gorham (1990) reported the role of 7H and 6H in modifying the Na^+ and K^+ contents in the leaves during salt stress, while Forster et al. (1990) found that barley chromosomes 4H and 5H had a positive effect on salt tolerance on the basis of growth parameters and yield components. The salt-tolerance of sea barleygrass (*H. marinum*), an extremely salt-tolerant species, was expressed in wheat–sea barleygrass (CS–H21) amphiploids (Islam et al. 2007) and the additive or interactive actions of chromosomes Hm2, Hm6 and Hm7 were observed in their addition lines (Raffi et al. 2010).

New wheat–barley addition lines were developed in Martonvásár between wheat line Mv9kr1 and barley cv. Igri (Molnár-Láng et al. 2000) and between wheat cv. Asakaze and barley cv. Manas (Molnár-Láng et al. 2012) in order to increase the allelic variation in the introgression lines. In this way, a set of disomic addition lines (2H, 3H, 4H, 6H and 7H) and several ditelosomic lines (3HS and 7HL) of Asakaze-Manas were produced (Molnár-Láng et al. 2012). Their salt-stress responses have not yet been characterized, but an earlier preliminary study showed that increasing salt concentrations caused a less pronounced decline in net photosynthesis in the Asakaze-Manas 7H addition line than in the parental variety Asakaze, suggesting that this line might be a good candidate for improving the salt tolerance of wheat (Dulai et al. 2010). The aim of the present study was to determine the salt stress responses of wheat cv. Asakaze and barley cv. Manas, together with an almost full set of addition lines carrying different barley chromosomes or chromosome arms. The particular aims were: (i) to compare the salt tolerance of the parental varieties with that of other wheat genotypes often used in breeding programmes; (ii) to investigate the effect of added barley chromosomes or chromosome segments on the salt tolerance of wheat–barley addition lines; (iii) to obtain deeper knowledge on the mechanism responsible for the greater salt tolerance of addition lines.

Materials and methods

Plant materials

The salt response was studied during germination and in young plants of the wheat–barley disomic addition lines 2H, 3H, 4H, 6H and 7H and the ditelosomic addition lines 3HS and 7HL developed from an Asakaze komugi (Asakaze; Japanese, facultative

wheat cultivar) × Manas (Ukrainian, six-rowed winter barley cultivar) hybrid (Molnár-Láng et al. 2000, 2012), and in the parental genotypes. The genetic stability of the addition lines was checked cytologically several times. Two other wheat genotypes, namely Mv9kr1, a winter wheat line originating from Martonvásár and used as parental genotype in other addition lines (Molnár-Láng et al. 1996), and Chinese Spring (CS), the wheat parental genotype of addition lines previously tested for salt tolerance (Colmer et al. 2006), were also used for comparison.

Germination test

Seeds (3 × 30 seeds of each genotype per treatment) were surface-sterilized in 10 % sodium hypochlorite for 15 min, rinsed twice in distilled water and germinated on wet filter paper containing 0, 100, 200 or 300 mM NaCl in Petri dishes for 3 days at room temperature. The percentage of germinated seeds and the length of root and coleoptyl were then determined.

Salt stress responses of young plants

Surface-sterilized seeds were germinated on wet filter paper in Petri dishes for 2 days at room temperature. Seedlings with similar root length were then grown in pots (10 plants/0.6 L pot) containing modified Hoagland solution (Pál et al. 2005) in a phytotron growth chamber (PGR15, Conviron) under a 16 h photoperiod at 120 µmol m^{-2} s^{-1} and 22/20 °C day/night temperature for 10 days. Salt stress was then induced by adding 100 mM NaCl to the Hoagland solution. After 7 days, the salt concentration was increased to 200 mM. The control plants continued to grow without salt treatment. The solutions were renewed every 2 days. The salt-induced decrease in growth was monitored by determining the root and leaf length on the 7th day of each salt treatment and the root and shoot weight (g/plant) at the end of the experiments.

Determination of physiological parameters

Salt-induced physiological changes were followed by monitoring changes in chlorophyll content, relative water content (RWC), chlorophyll-*a* fluorescence parameters, gas exchange activity and the osmotic potential of the leaves, and by determining the proline, Na and K contents of both roots and leaves.

A SPAD-502 chlorophyll meter (Spectrum Technologies, Plainfield, IL, USA) was used to determine the chlorophyll content of intact leaves. RWC was determined as RWC % = (FW−DW)/(SW−DW) × 100, where FW is fresh weight, SW is water-saturated weight and DW is oven-dried weight at 80 °C for 48 h.

The gas exchange was analysed on intact leaves with a Ciras 2 Portable Photosynthesis System (Amesbury, USA) using a narrow leaf area (2.5 cm^2) chamber. The parameters, CO_2 assimilation rate (*Pn*), stomatal conductance (g_s), intracellular CO_2 concentration (C_i) and transpiration (*E*) were measured at the steady-state level of photosynthesis using a CO_2 level of 380 µL l^{-1} and light intensity of 300 µmol m^{-2} s^{-1}.

Chlorophyll-*a* fluorescence was measured on intact detached leaves using a PAM-2000 chlorophyll fluorometer (Walz, Effeltrich, Germany). The *Fv*/*Fm* parameter, indicating the maximal quantum efficiency of PS II, was determined on leaves dark-adapted for 15 min. Photosynthesis was then induced by continuous illumination of the leaves at 200 µmol m^{-2} s^{-1} for 15 min. The parameters $\Delta F/F_m'$, (which estimates the actual quantum yield of PS II at given light intensity), qP (photochemical quenching parameter, related to the fraction of open reaction centres in PS II), and NPQ (non-photochemical quenching parameter, which reflects the heat dissipation of excess excitation energy) were calculated at the steady-state level of photosynthesis according to the nomenclature described by van Kooten and Snell (1990).

The osmolarity of leaf sap from salt-stressed and control leaves and also from water-saturated leaves (kept between wet filter papers for 4 h) was measured using an Osmomat 030, freezing-point osmometer (Gonotech GmbH, Berlin, Germany), and osmotic potential (Ψ_π) values were calculated as described by Bajji et al. (2001). Osmotic adjustment (OA) was calculated as the difference between the osmotic potentials measured in control and salt-stressed plants. Active osmotic adjustment (AOA) was defined as the difference between osmotic potentials at full turgor in control and salt-stressed leaves, while passive osmotic adjustment (POA) was defined as POA = OA−AOA as described by Girma and Krieg (1992) and Boussadia et al. (2013).

The proline content was determined according to Bates et al. (1973). The amounts of Na and K were determined from air-dried leaf samples (0.5 g sample^{-1}) using the inductively coupled plasma-atomic

emission spectrometry method (ICP-AES, Jobin–Yvon Ultima 2 sequential instrument) after microwave Teflon bomb digestion with cc. HNO_3 + HCl (Anton et al. 2012).

Statistical analysis

The results were obtained in two independent series of experiments. In each experiment, five pots (10 plants/pot) of each genotype were used for the control (without salt treatment) and five for the salt treatment. Samples were collected from each pot and the measurements were performed on at least 3 biological replicates in each experiment. Differences between treatments or between the genotypes within each treatment were determined by means of Tukey's post hoc test (SPSS 16.0).

Results

Germination under salt conditions

In the first experiment three wheat genotypes (CS, Mv9kr1 and Asakaze), one barley genotype (cv. Manas), five disomic addition lines (Asakaze-Manas 2H, 3H, 4H, 6H and 7H) and the Asakaze-Manas 3HS and 7HL ditelosomic addition lines were exposed to 0, 100, 200 or 300 mM NaCl during the germination phase. Under control conditions (0 mM NaCl) the germination % varied between 94 and 100 %, which was not significant (Table 1). 100 mM salt only reduced the germination % slightly in the case of Mv9kr1 and Asakaze-Manas 3H. Raising the salt concentration (200 mM) inhibited the germination in all the genotypes tested. Barley cv. Manas exhibited the highest germination %, while it was the lowest in wheat cv. Mv9kr1. The germination was better in wheat cv. Asakaze than in Mv9kr1 or CS, but was significantly lower than in barley cv. Manas. The salt-induced reduction in germination % and the differences between the lines were more pronounced when 300 mM NaCl was applied. A comparison of the addition lines revealed that the addition of chromosomes or chromosome segments reduced the germination rate under salt conditions in addition lines 2H, 3H and 3HS, and only at 300 mM NaCl in addition line 6H, while in Asakaze-Manas 7H and 7HL the germination was significantly higher than in the wheat parent Asakaze (Table 1).

Growth parameters under salt conditions

In this experiment, 10-day-old plants were exposed to salt concentrations, which were increased at weekly intervals, while the control plants were grown without added NaCl. The root length remained significantly lower in barley cv. Manas than in the other genotypes throughout the experiment, irrespective of the treatments (Fig. 1a). The 100 mM salt treatment only had a slight effect on root growth, and the greatest reduction in root length was observed in Mv9kr1 and Asakaze-Manas 6H (Fig. 1a, right panel). In contrast, the 100 mM salt treatment inhibited shoot growth in most of the genotypes (Fig. 1b, right panel). The least reduction in shoot length was observed in barley cv. Manas, wheat cv. Asakaze and the 7H and 7HL addition lines. The 200 mM salt treatment reduced both root and shoot growth in almost all the genotypes as compared to the control plants, with the smallest decrease in Manas and the addition lines 7H and 7HL (Fig. 1a, b). The salt-induced decrease in growth was also manifested in the lower root and shoot weights determined at the end of the experiment (Table 2). The reduction was more severe in the shoots than in the roots. The least reduction in biomass was observed in both the roots and shoots of Manas and the addition lines 7H and 7HL. The different responses of root and shoot weights to salt were reflected in the change in the shoot/root ratio. As presented in Table 2, the genotypes used in these experiments did not differ significantly for this parameter under control conditions, but a substantial decrease was observed under salt-stressed conditions, especially in genotypes CS, Mv9kr1, Asakaze-Manas 2H and 6H. There was no change in the shoot/root ratio in barley cv. Manas, and only a slight decrease was observed in addition lines 7H and 7HL (Table 2), suggesting that barley plants were more efficient in maintaining shoot biomass production. The growth parameters also revealed that, although the root length was the lowest in barley cv. Manas under control conditions, this difference was not manifested in the root weight, since the density of the roots compensated for the short length (Table 2).

Relative water content and chlorophyll content

The decrease in the relative water content of the leaves is one of the most obvious symptoms of osmotic stress. Exposure to a week each of 100 and 200 mM salt stress

Table 1 Germination percentage (%) of genotypes germinated at several (0, 100, 200 and 300 mM) NaCl concentrations. (AK: Asakaze)

Genotypes/lines	Germination percentage (%)			
	0 mM	100 mM	200 mM	300 mM
CS	100a	100a	83 ± 3.3b	53 ± 6.2c
Mv9kr1	97 ± 3.0a	90 ± 1.4c	48 ± 4.8d	18 ± 3.3e
AK	100a	100a	88 ± 4.5ab	77 ± 2.1b
AK-Manas 2H	94 ± 4.6a	93 ± 4.7bc	68 ± 2.4c	52 ± 7.1c
AK-Manas 3H	97 ± 2.5a	90 ± 1.4c	80 ± 4.8b	38 ± 2.4d
AK-Manas 3HS	94 ± 4.0a	91 ± 1.4c	55 ± 5.0d	35 ± 4.2d
AK-Manas 4H	97 ± 3.4a	96 ± 1.2ab	85 ± 4.7b	73 ± 6.7b
AK-Manas 6H	96 ± 5.0a	95 ± 1.8b	84 ± 3.8b	50 ± 6.4c
AK-Manas 7H	97 ± 4.2a	95 ± 2.8b	92 ± 4.0ab	80 ± 1.5ab
AK-Manas 7HL	97 ± 3.4a	96 ± 1.4ab	95 ± 4.0a	85 ± 3.3a
Manas	100a	100a	96 ± 3.8a	85 ± 4.5a

Different letters indicate statistically significant differences at $P < 0.05$, using Tukey's post hoc test

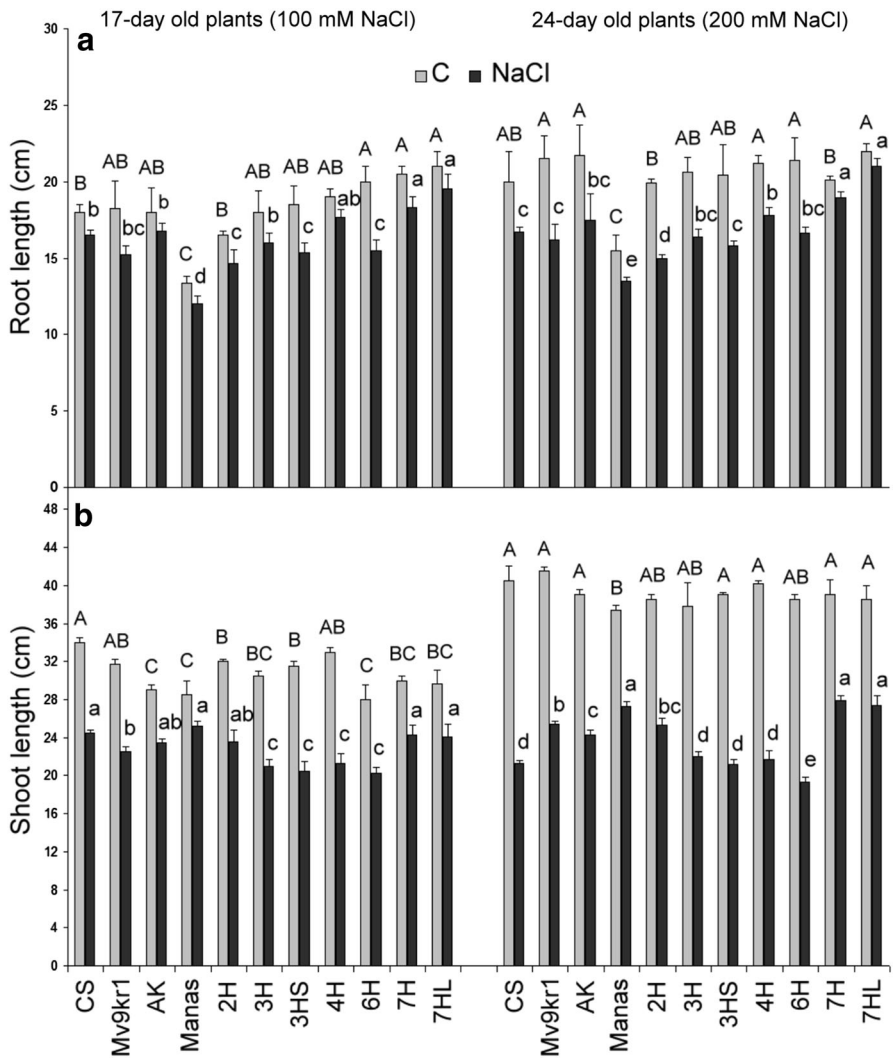

Fig. 1 Root (**a**) and shoot (**b**) length of plants grown in hydroponic solution with or without 100 mM (*left panel*) or 200 mM (*right panel*) NaCl. Data represent mean ± standard deviation of five plants per pot in each treatment. Different letters indicate statistically significant differences between the genotypes in control (*uppercase*) and salt-treated plants (*lowercase*) at $P < 0.05$, using Tukey's post hoc test. (AK: Asakaze)

Table 2 Root and shoot weight (g/plant) of plants grown in hydroponic solution with (NaCl) and without (C) salt treatment; the shoot/root ratio in control and salt-treated plants. (AK: Asakaze)

Genotypes/lines	Root (g/plant)		Shoot (g/plant)		Shoot/root ratio	
	C	NaCl	C	NaCl	C	NaCl
CS	1.12 ± 0.13a	0.45 ± 0.06ab	2.3 ± 0.3a	0.41 ± 0.06c	2.05ab	0.91e
Mv9krl	1.01 ± 0.09ab	0.43 ± 0.03ab	1.84 ± 0.14b	0.46 ± 0.03c	1.82c	1.07de
AK	0.94 ± 0.13ab	0.39 ± 0.03b	1.98 ± 0.15ab	0.60 ± 0.05b	2.10a	1.54c
AK-Manas 2H	1.10 ± 0.06a	0.48 ± 0.04a	2.10 ± 0.11a	0.44 ± 0.08c	1.91b	0.92e
AK-Manas 3H	0.97 ± 0.06ab	0.39 ± 0.02b	2.12 ± 0.06a	0.51 ± 0.09bc	2.18a	1.30d
AK-Manas 3HS	1.07 ± 0.04a	0.26 ± 0.04c	2.10 ± 0.14a	0.41 ± 0.08c	1.96b	1.57c
AK-Manas 4H	1.04 ± 0.04a	0.39 ± 0.04b	2.09 ± 00.9a	0.56 ± 0.04b	2.01b	1.44cd
AK-Manas 6H	1.02 ± 0.03a	0.47 ± 0.01ab	2.04 ± 0.04a	0.44 ± 0.03c	2.00b	0.94e
AK-Manas 7H	0.86 ± 0.06b	0.45 ± 0.03ab	1.97 ± 0.05ab	0.82 ± 0.08a	2.29a	1.82b
AK-Manas 7HL	1.04 ± 0.05a	0.51 ± 0.02a	2.10 ± 0.004a	0.85 ± 0.04a	2.02b	1.67bc
Manas	0.88 ± 0.14ab	0.47 ± 0.02a	1.81 ± 0.05b	0.95 ± 0.07a	2.05ab	2.02a

Weight data were determined at the end of the experiment. Data represent mean ± standard deviation of five pots each (10 plants/pot) for control and for salt-treated plants. Different letters indicate statistically significant differences at $P < 0.05$, using Tukey's post hoc test

reduced the RWC of the leaves from above 90 % (control) to 70–80 % in most of the genotypes. The lowest values were measured in CS and Mv9kr1, while the highest RWC was detected in barley cv. Manas, which remained at almost 90 %. While the majority of addition lines performed similarly to the parental wheat Asakaze, the addition lines 7H and 7HL showed a less pronounced decrease in RWC than the other genotypes (Fig. 2a).

Chlorosis is one of the most obvious stress symptoms, especially when the stress is accompanied by oxidative damage. Under control growth conditions barley cv. Manas, wheat cv. Mv9kr1 and addition line 6H showed slightly higher chlorophyll content than the other genotypes (Fig. 2b). While treatment with 100 mM NaCl did not significantly reduce the chlorophyll content (data not shown), a subsequent week at 200 mM caused a more pronounced decrease in the SPAD values, indicating a decrease in leaf chlorophyll content (Fig. 2b). The smallest decrease was detected in barley cv. Manas, although the difference between Manas and the other genotypes was not as obvious as for other stress parameters, such as the decrease in RWC or root growth.

Gas exchange parameters

The net photosynthesis (*Pn*) was only slightly higher in barley cv. Manas than in the other genotypes under control conditions, although the differences were not substantial (Fig. 3a). Since the correlation between the SPAD values and the net photosynthesis values was not significant (data not shown), this relatively high photosynthesis can only be partly explained by the higher chlorophyll content.

Salt-induced osmotic stress causes rapid stomata closure and a decrease in photosynthetic activity. 100 mM salt treatment for 1 week significantly reduced the net photosynthesis in the majority of genotypes, with the exception of Manas and addition lines 6H, 7H and 7HL (Fig. 3a). An additional week of exposure to 200 mM NaCl decreased this parameter more intensively, and the differences between the genotypes became more pronounced. While the decrease in *Pn* was more pronounced in Mv9kr1, Asakaze-Manas 2H, 3H, 3HS and 4H than in wheat cv. Asakaze, even higher values were recorded for barley cv. Manas and addition lines 6H, 7H and 7HL, indicating that these genotypes can maintain high photosynthetic activity under salt stress conditions.

The changes in stomatal conductivity (g_s) showed a pattern similar to those in net photosynthesis (Fig. 3b), indicating a close ($R^2 = 0.85$) correlation between stomatal closure and photosynthetic activity in the leaves. These results suggest that the higher level of net photosynthesis in Manas under control and saline conditions and in genotypes Asakaze-Manas 6H, 7H

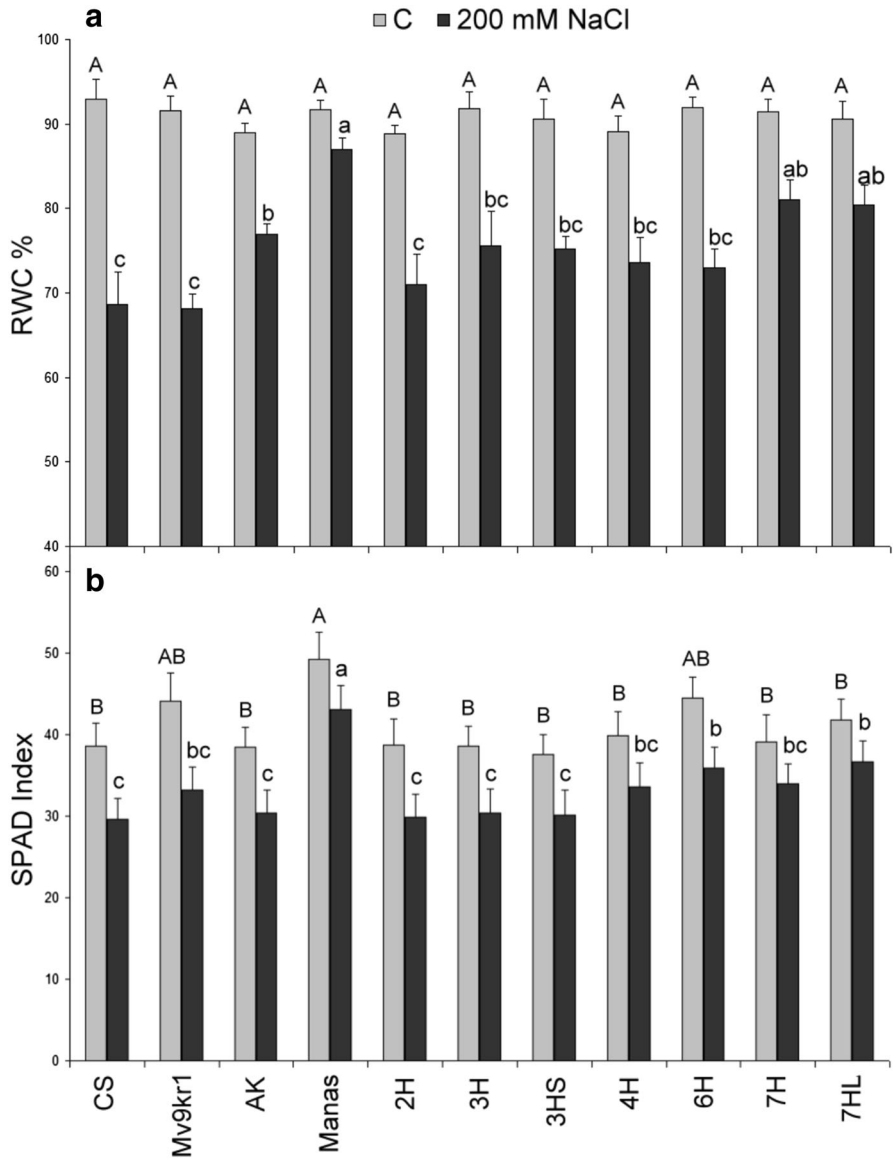

Fig. 2 Relative water content (%) (**a**) and chlorophyll content as indicated by SPAD index (**b**) in leaves of 24-day-old plants in the absence and presence of NaCl treatment. Data represent mean ± standard deviation; $n = 8$ for RWC and 20 for determination of SPAD index in each treatment. Different letters indicate statistically significant differences between the genotypes in control (*uppercase*) and salt-treated plants (*lowercase*) at $P < 0.05$, using Tukey's post hoc test. (AK:Asakaze)

and 7HL can be explained by the lower stomatal closure (Fig. 3b).

Chlorophyll-a fluorescence induction

The chlorophyll-*a* fluorescence induction technique provides a sensitive method to follow stress-induced changes in photosynthetic electron transport activity. The 100 mM salt treatment did not induce severe changes in most of the chlorophyll-*a* fluorescence parameters, indicating that the photosynthetic electron transport processes around PSII are less sensitive to salt-induced changes than CO_2 assimilation. Consequently, the results obtained for chlorophyll-*a* fluorescence parameters are only presented for the 200 mM salt treatment in the online resource (1). The F_v/F_m chlorophyll-*a* fluorescence parameter,

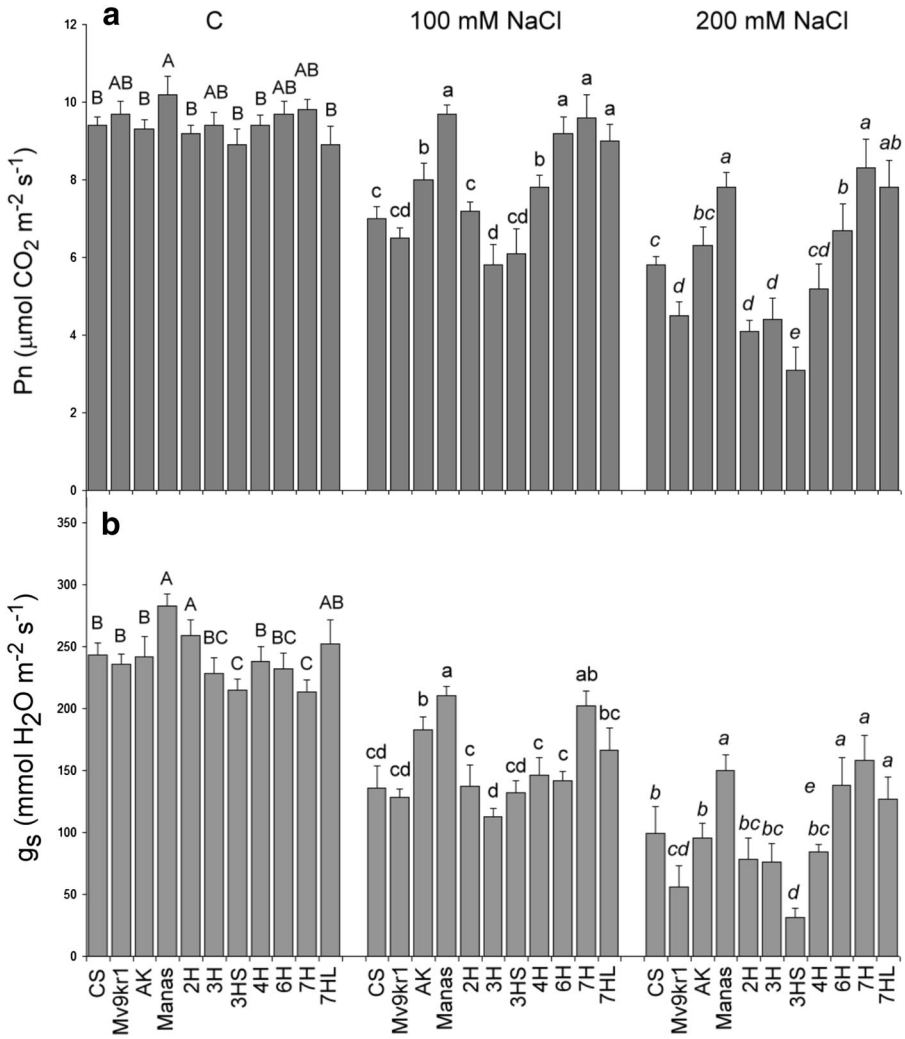

Fig. 3 Photosynthetic activity, Pn (**a**) and stomatal conductance (g_s) (**b**) in leaves treated with salt (100 or 200 mM) or without salt treatment. The parameters were determined at the steady-state of photosynthesis using 300 μM m^{-2} s^{-1} actinic light intensity. Values are mean ± standard deviation of five replicates per treatment. Different letters indicate statistically significant differences between the genotypes in control (*uppercase*) and salt-treated plants (*lowercase*) at $P < 0.05$, using Tukey's post hoc test. (AK: Asakaze)

which represents the maximum quantum yield of Photosystem II (PSII), was around 0.8 in the control leaves, a value typical of healthy plants. The elevated (200 mM) salt concentration caused significant reduction in F_v/F_m only in wheat cv. Mv9kr1 and in the Asakaze-Manas 2H addition line (online resource 1a). The response of the actual quantum efficiency of PSII, represented by $\Delta F/F_m'$, is typically more sensitive than that of F_v/F_m. The 200 mM NaCl treatment caused a substantial decrease in $\Delta F/F_m'$ in almost all the genotypes, with the exception of Manas and the Asakaze-Manas 7H and 7HL lines (online resource 1b). The photochemical quenching (*qP*) parameter, representing the ratio of open PSII centres, showed a pattern similar to that of the actual quantum yield (online resource 1c). However, NPQ behaved differently: although there were some variations in the control plants, the differences were not substantial. Exposure to 200 mM salt caused an increase in all the genotypes. The smallest changes occurred in barley cv. Manas, while the highest values of NPQ were found for Asakaze-Manas 4H (online resource 1d).

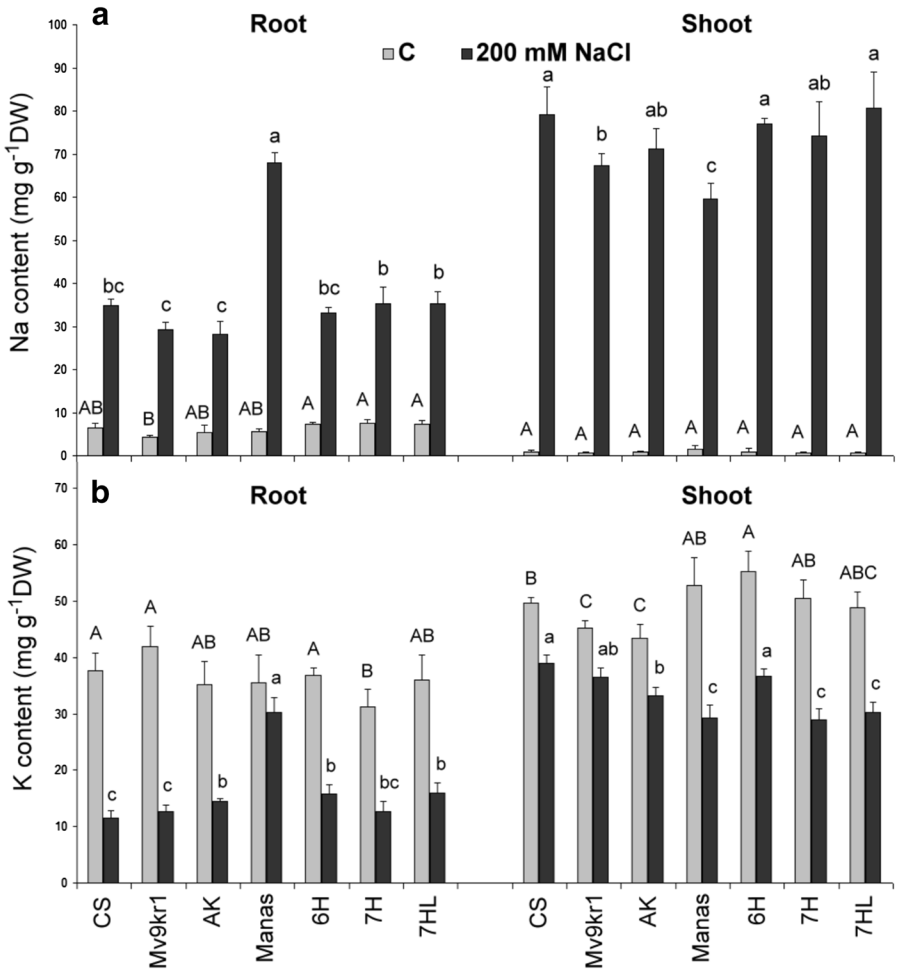

Fig. 4 Na (**a**) and K (**b**) contents of roots (*left*) and shoots (*right*) grown in hydroponic solution with or without NaCl. Values were determined from air-dry samples collected at the end of the experiments, and the data are mean ± standard deviation of three replicates of each treatment. Different letters indicate statistically significant differences between the genotypes in control (*uppercase*) and salt-treated plants (*lowercase*) at $P < 0.05$, using Tukey's post hoc test. (AK: Asakaze)

Mineral composition, osmotic adjustment and proline content

On the basis of the physiological responses to salinity, it seems that barley cv. Manas and Asakaze-Manas addition lines carrying chromosome 7 from Manas performed better under saline conditions than the wheat parental line Asakaze. Furthermore, although the addition line 6H exhibited elevated salt tolerance for certain parameters such as germination % and growth, this line also showed better net photosynthesis than the parental wheat cv. Asakaze under stress conditions. In order to reveal the biochemical mechanisms playing a role in improved salt tolerance, the mineral composition, osmotic potential and proline content were also determined in these genotypes.

As expected, there was no substantial difference between the Na contents of the various control plants, while high salinity substantially increased the Na contents in both the roots and shoots (Fig. 4a). A comparison of the genotypes showed that the Na content in Manas after exposure to high salt concentration was significantly higher than in the other genotypes in the roots, but was the lowest in the shoots, indicating the greater accumulation of Na in the roots, but restricted transport to the shoots. The K content significantly decreased in both the roots and shoots of plants treated with NaCl: however, this

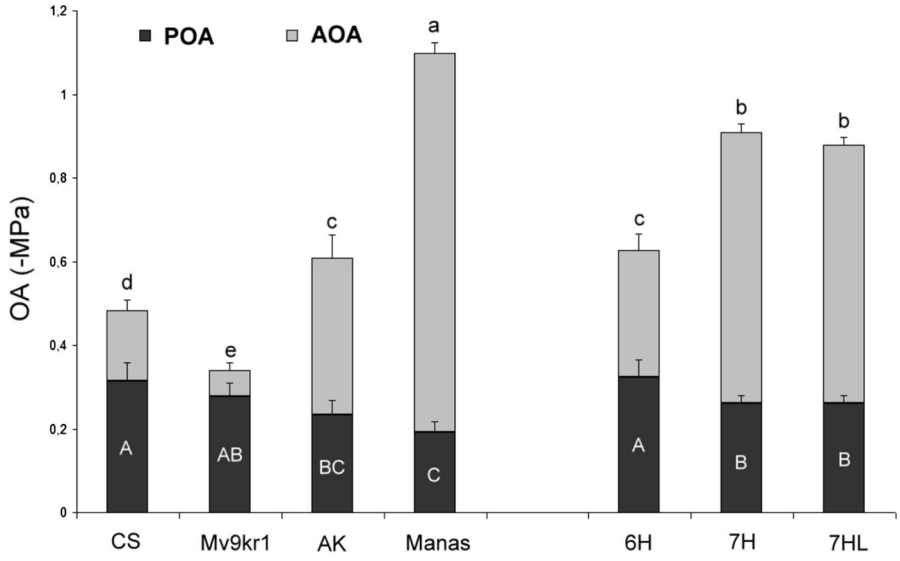

Fig. 5 Osmotic adjustment (*OA*) in leaves of wheat (*CS, Asakaze* and *Mv9kr*1), barley (cv. *Manas*) and *Asakaze-Manas introgression lines* 6H, 7H and 7HL. *OA* was calculated as the difference between osmotic potential values measured in control and salt-stressed leaves. Active (*AOA*) and passive (*POA*) osmotic adjustment was discriminated as described in "Materials and methods" section. (AK: Asakaze)

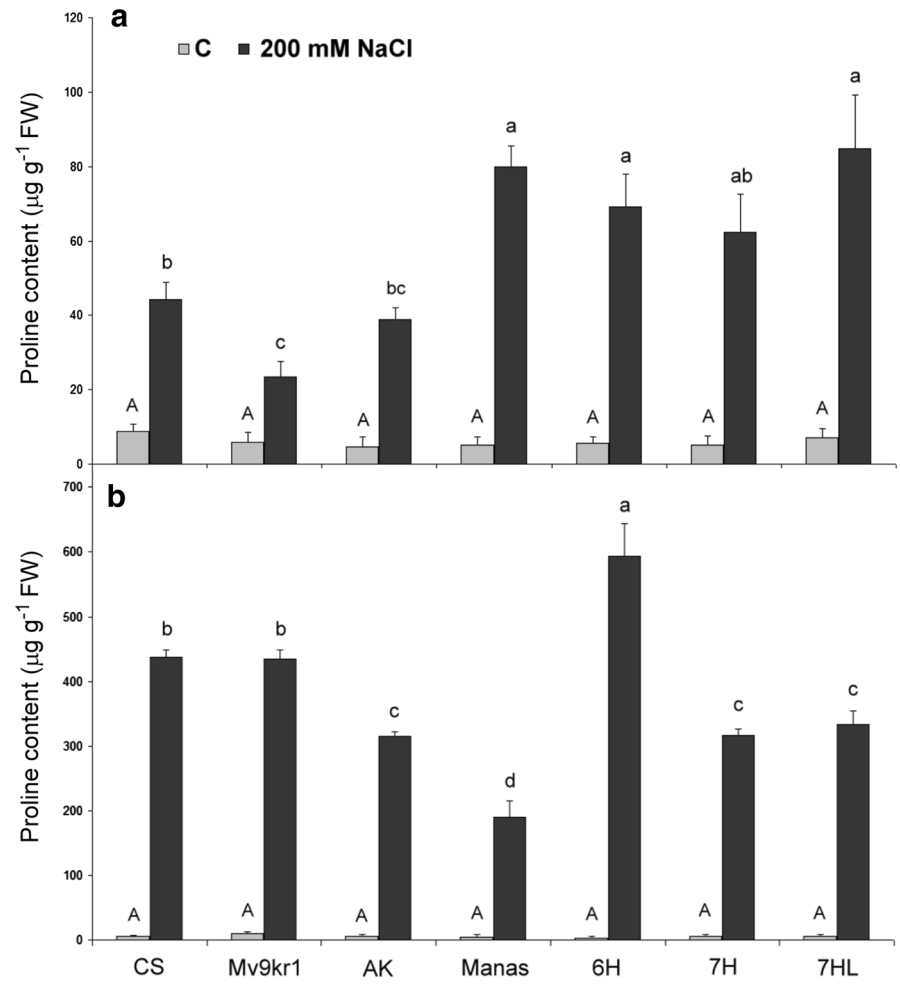

Fig. 6 Effect of salinity treatment on proline content in roots and shoots of wheat (*CS, Asakaze,* and *Mv9kr*1), barley (cv. *Manas*) and *Asakaze-Manas introgression lines*. Samples were collected at the end of the experiments, and the data are mean ± standard deviation of three biological replicates for each treatment. Different letters indicate statistically significant differences between the genotypes in control (*uppercase*) and salt-treated plants (*lowercase*) at $P < 0.05$, using Tukey's post hoc test. (AK: Asakaze)

decrease was less pronounced in the roots of barley cv. Manas (Fig. 4b). The Na and K levels of the disomic addition lines 6H and 7H and the ditelosomic line 7HL were closer to those of the wheat parent Asakaze than to barley cv. Manas, indicating that the redistribution of the ionic compounds causing salt stress was not modified substantially in the addition lines.

Salinity modifies the osmotic potential of cells not only due to the decrease in water content but also through the synthesis of osmolites. As seen in Fig. 5, the stress-induced increase in the osmotic potential was the lowest in wheat cv. Mv9kr1, where there was a high proportion of passive osmotic adjustment (POA). This was followed by CS and Asakaze. The osmotic adjustment was similar to that of wheat cv. Asakaze in addition line 6H, but it was substantially higher in addition lines 7H and 7HL. Active osmotic adjustment (AOA) was dominant in these genotypes, as in barley cv. Manas, which had the highest increase in osmotic potential. These results suggest that the improved level of salt tolerance in Manas, Asakaze-Manas 7H and 7HL could be explained by their better ability to adjust to osmotic pressure.

Proline is one of the osmolites typically accumulated during salt stress. Under control growth conditions the proline contents were very low in both the roots and shoots of all the genotypes tested. Exposure to 200 mM dramatically increased the proline level, and this increase was more pronounced in the leaves than in the roots (Fig. 6 a, b). In the roots the proline content increased to the least extent in Mv9kr1 and to the greatest extent in Manas and the Asakaze-Manas 7H and 6H additions, indicating that proline accumulation in the roots may contribute to their acclimation to saline conditions. In the leaves, however, the smallest increase occurred in Manas, while the highest proline content was measured in Asakaze-Manas 6H.

Discussion

Wheat is a moderately salt-tolerant cereal, while barley has greater salt tolerance (Munns et al. 2011), making it a potential candidate for improving the salt tolerance of wheat.

The present results showed that, among the wheat genotypes frequently used for interspecific hybridization, such as CS, Mv9kr1 and Asakaze (Molnár-Láng et al. 2014), the genotype Asakaze possessed relatively high salt tolerance. Under salt stress conditions, Asakaze exhibited less reduction in germination (%) and growth, especially in the case of shoot growth, and retained higher leaf water content and photosynthetic activity (*Pn*) than Mv9kr1 or CS. However, the results showed that barley cv. Manas had even better salt tolerance than Asakaze, both during germination and in early developmental stages. Rather than excluding salt, barley cv. Manas takes up Na and accumulates it in the roots, while restricting its transport to the shoots. Consequently, the root elongation was retarded to a similar extent in both wheat and barley, while the shoot growth was less affected by salt, leading to a higher shoot/root ratio in barley than in wheat. Since the roots of barley seemed healthy and retained their K content, it can be assumed that Na was partitioned in a safe cell compartment in the roots, possibly in the vacuoles. The high level of proline accumulated in the root may also participate in balancing the osmotic pressure and protecting the roots against the damage induced by salt. In addition, the Na^+ exclusion mechanisms operating in the shoot may also contribute to the less extensive damage to the leaves in barley cv. Manas (as indicated by the SPAD values) and to the higher photosynthetic activity and RWC content in the leaves than was recorded for wheat varieties.

A further question was whether the better salt tolerance of barley cv. Manas could be transferred to wheat, and whether the salt tolerance mechanism of Manas was expressed in the wheat background in any of the addition lines. A preliminary experiment showed that the Asakaze-Manas 7H addition line, like the parental barley cv. Manas, was able to retain its CO_2 fixation rate during salt stress with relatively high g_s (Dulai et al. 2010). The present study also proved that this addition line is characterised by better salt tolerance, as indicated by the high germination rate, limited growth reduction and high shoot/root ratio, and by the retention of photosynthetic activity and water content under salt stress conditions. In addition, elevated salt tolerance was also observed in the Asakaze-Manas 7HL ditelosomic line, indicating that the long arm of chromosome 7H may be responsible for the improved salt tolerance. The role of 7H and 6H in the salt tolerance of addition lines originating from CS wheat and Betzes barley was also proved by Gorham (1990), who found that it was related to the modification of Na^+ and K^+ contents in the leaves. However, a

low (60 mM) Na concentration was used in this work, possibly due to the low salt tolerance of CS. When moderate salt concentrations (175–200 mM) were applied to these lines, the decreases in growth and yield were indicative of enhanced salt tolerance, though the Na content was not determined (Forster et al. 1990). In the present work, greater salt tolerance was observed for addition lines 7H and 7HL in the 100 and 200 mM NaCl treatments, while the Na and K contents in these lines resembled those of the parental wheat line rather than those of barley cv. Manas, suggesting that the increased salt tolerance of these genotypes was probably not related to the different Na^+ transport mechanisms observed in barley Manas.

Osmotic stress is an early effect of salinity, causing physiological water deficit in all plant organs (Chaves et al. 2009). One strategy used by plants to avoid water deficiency is the development of long roots. Although the addition lines Asakaze-Manas 7H and 7HL had slightly longer roots than the majority of the genotypes tested in the present work, the high salt tolerance of the donor genotype, barley cv. Manas, could not be explained in this way, because it had the shortest roots under both control and saline conditions. It should be mentioned, however, that the root weight of barley cv. Manas was among the highest, indicating that the short length was compensated for by higher density. Furthermore, the fact that the shoot/root weight ratio remained high even under saline condition in addition lines 7H and 7HL, as in barley cv. Manas suggests that the avoidance of osmotic stress is not the main strategy of this variety.

The effects of osmotic stress on photosynthesis range from the restriction of CO_2 diffusion into the chloroplast (via the limitation of stomatal opening, mediated by shoot- and root-generated hormones, and of the mesophyll transport of CO_2) to alterations in leaf photochemistry and carbon metabolism (Lawlor and Cornic 2002; Munns 2002). The present results showed that the Asakaze-Manas 7H and 7HL addition lines were able to retain their photosynthetic activity together with low stomatal closure, as indicated by the high g_s values (Fig. 3). Nevertheless, they were able to preserve their water content (Fig. 2a). The high quantum yield of PSII and the low values of NPQ (online resource 1) also suggest that these genotypes managed to maintain a balanced homeostasis, allowing the photosynthetic apparatus to function as efficiently as possible. The better performance of these genotypes under saline conditions was probably mainly due to the improvement of osmotic adjustment, as demonstrated by the elevated osmotic potential in salt-treated Asakaze-Manas 7H and 7HL addition lines, similarly to that recorded for barley cv. Manas. These results are in accordance with earlier results where QTL analysis revealed that a homoeologous region on chromosome 7H was involved in osmotic adjustment in barley (Teulat et al. 1998). Although the synthesis of certain osmotically active compounds could be an important part of the salt tolerance strategy in these genotypes, proline is not the main factor in the leaves. Nevertheless, it may be important in the root, as elevated proline content was detected in the Asakaze-Manas 7H and 7HL addition lines and also in the parental barley cv. Manas.

The effect of barley chromosomes on the salt stress response of the other additions was not so evident. Earlier studies using disomic addition lines originating from wheat cv. CS and barley cv. Betzes or *Hordeum chilense* revealed significant interactions between the genotype and the salt concentration, indicating that specific chromosomes may have either positive (especially 4H and 5H or $4H^{ch}$ and $5H^{ch}$) or negative consequences for salt tolerance (Forster et al. 1990). These authors also concluded that chromosomes 5H and 6H carried genes for vigour (Forster et al. 1990). In the present experiments, addition line 6H showed relatively high net assimilation under saline conditions, despite the reduced germination rate and low biomass production as also found by Forster et al. (1990). It is possible that the slightly higher chlorophyll content and the high proline level may also contribute to the higher photosynthetic ability of Asakaze-Manas 6H at high salt concentrations. Besides its osmotic role, proline may also play a general stress-signalling role during osmotic stress (Szabados and Savouré 2009). Nevertheless, this genotype was unable to cope with salt stress as efficiently as either the donor species barley cv. Manas or addition lines 7H and 7HL.

In spite of the fact that a gene (*HKT1;5*) encoding a Na^+-selective transporter was identified on chromosomes 4H and was recently used to produce salt-tolerant durum wheat showing increased salt tolerance, with yield increases of 25 % on saline soil (Munns et al. 2012), the root and shoot Na^+ content was not modified significantly in Asakaze-Manas 4H addition line compared to the parental wheat line. The influence of chromosome 4H on the salt tolerance of the CS–Betzes addition line, reported by Forster et al. (1990), was not

reflected in the present work either. However, their results were based on agronomic properties, especially growth and yield components, which are affected by a number of genetic and environmental factors. Handley et al. (1994) found that barley chromosome 4H was able to increase the water use efficiency in wheat addition lines. A similar conclusion was obtained when the wheat Mv9kr1–Betzes 4H(4D) substitution line was exposed to mild osmotic stress induced by polyethylene glycol, PEG (Molnár et al. 2007). The present experiments showed that under saline conditions the 4H Asakaze–Manas addition line was characterised by high NPQ, a mechanism which also regulates photosynthetic electron transport processes (Horton and Ruban 2005); however, this was not manifested as a higher level of salt tolerance.

Among the other genotypes, addition lines 2H, 3H and 3HS exhibited the worst physiological responses to salt stress for the majority of stress-related parameters, namely germination, biomass production, RWC, gas exchange and the quantum efficiency of PSII. The reason for the decrease in salt tolerance in several of the addition lines used either in the present work or in other experiments (Forster et al. 1990) is still unknown. Similar phenomena have been described for pathogen resistance, which may be suppressed by the presence of alien genes (Chen et al. 2013). Suppressors acting on resistance genes from an alien genetic background have been identified in various wheat chromosomes. For example, a specific suppressor for leaf rust resistance gene *Lr*23 was located on chromosome arm 2DS (Nelson et al. 1997), and a gene suppressing the rye-derived powdery mildew resistance genes *Pm*8 and *Pm*17 in common wheat was localized on chromosome 7D (Zeller and Hsam 1996). However, further experiments will be required to reveal the interactive effects of barley and wheat chromosomes on salt tolerance.

In conclusion, the present results suggest that although the salt tolerance of Asakaze is relatively high compared with that of other wheat genotypes, including CS, a genotype frequently used for interspecific hybridization, it can be improved by the introgression of chromosome 7H from barley cv. Manas or by the long arm, 7HL. The elevated salt tolerance of the 7H and 7HL introgression lines is manifested in germinating seeds as well as in plants with high photosynthetic capacity. Although the salt-induced Na accumulation differed in the wheat and barley parents and the Na content of addition lines 7H and 7HL more closely resembled that of the parental wheat line than that of barley cv. Manas, the elevated osmotic adjustment of the barley parent was manifested in Asakaze-Manas lines 7H and 7HL, and may thus have contributed to the retention of growth and photosynthetic activity even under salt stress conditions. Further research will be conducted to reveal the detailed mechanisms of the improved salt tolerance of these addition lines.

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